Prognostic Significance of Wilms Tumor 1 Gene in Childhood Acute Lymphoblastic Leukemia

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ABSTRACT

Wilms tumor 1 (WT1) gene is a tumor supressor gene, expressed in malignant and normal hematopoietic progenitor cells. Prognostic significance of this gene in childhood acute lymphoid leukemia (ALL) is not clear. We evaluated the presence of WT1 expression in bone marrow samples of 28 children with de novo ALL at diagnosis by two step RT-PCR. Expression of WT1 gene was detected in 78.5% of patients. There was no correlation between WT1 gene expression and age, sex, FAB type, leukocyte count, and presence of t(4;11) and t(9;22). All patients were treated with modified BFM 86 protocol. There was no difference in the complete remission (CR) rate between WT1 positive and negative patients. Event free survival (EFS) and overall survival (OS) of WT1 positive and negative patients were also not significant. We conclude that expression of WT1 gene is not associated with specific characteristics of ALL blast cells and is not a prognostic factor for CR, remission duration and overall survival.

Key Words: Acute lymphoblastic leukemia, Childhood, Prognosis, Wilms tumor 1 gene.

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INTRODUCTION

Wilms tumor 1 gene is a tumor supressor gene, located in chromosome 11p13[1-4]. WT1 gene expression has been shown in embrional renal tissue, gonads, mesothelium, uterine decidua, lung and thymus[1,4,5]. Expression of WT1 gene in leukemia derived cell lines and mononuclear cells from patients with acute leukemia has been demonstrated[1,6,7]. It has been suggested that it has prognostic value for adult acute leukemia[4]. On the other hand, prognostic significance of WT1 gene expression has not been supported by other studies, claiming that it is not a prognostic factor for acute myeloid leukemia[6]. Prognostic significance of WT1 gene expression in childhood ALL is not studied widely. We investigated the prognostic significance of WT1 gene expression for CR, EFS and OS, and relationship between classic prognostic factors and WT1 expression in childhood acute lymphoblastic leukemia.

MATERIAL and METHODS

Patients: Between September 1995 and July 1996, 28 patients with de novo ALL were evaluated for WT1 gene expression at diagnosis and at the end of the induction therapy. Patients characteristics are shown in Table 1. Leukemia was classified according to the French-American-British (FAB) criteria after examination of bone marrow aspirates stained by wright technique. In addition, immune phenotyping was performed in each case. All patients were treated with CCG 105 protocol, including daunorubicin 25 mg/m^2 and vincristine 1.5 mg/m^2 on days 1, 8, 15, 21, L-asparaginase 10.000 U/m^2 on days 3, 5, 7, 10, 12, 14, 17, 19, 21 and prednisolon 60 mg/m^2 on days 1 to 21 in the induction period. All patients who achieved CR received one consolidation course including cytosine arabinoside, cyclophosphamide, and 6-mercaptopurine.

Qualitative Polymerase Chain Reaction: Bone marrow aspirates were obtained from all patients at diagnosis and the end of the induction therapy. Mononuclear cells were separated by density gradient centrifugation using ficoll-hypaque. Total RNA was extracted from 10^7 cells according to acid guanidum thiocyanate-phenol-chloroform method[8]. RNA was dissolved in diethylpyrocarbonate-treated water and quantitated spectrometrically with absorbance at 260 nm. One microgram of total RNA in 15.5 L of diethylpyrocarbonate-treated water was heated at 65 C for 5 minutes and then mixed with 14.5 L of RT buffer (Gibco-BRL, 18064-014) containing 600 U of monkey murine leukemia virus reverse transcriptase (Gibco BRL-18064-01489), 750 ng of random hexamer (Sigma H.0268) and 40 U of RNAse inhibitor (Promega 5726909) was incubated at 37 °C for 90 minutes, heated at 70^{o}C for 20 minutes, and then stored at -20^{o}C until use. PCR were performed for 30 cycles with a DNA thermal cycler (Perkin Elmer-Cetus DNA Thermal Cycler 480) under the following conditions: denaturation at 94 C for 1 minute, primer annealing at 64^{o}C for 1 minute, and then chain elongation at 72^{o}C for 2 minutes. When the densitometric units of PCR products of the first round were less than 500, the second round PCR was performed with the nested internal primers in a reaction solution containing 2.5 L of first round PCR products. PCR products were quantitated as described previously[9]. PCR products derived from 20 ng of total RNA (in second round 1 g) were separated in 2 % agarose gel containing 0.05 g/mL ethidium bromide, and photographed with transluminator via computer (Collage 2.0 program, Collage Image Information Extraction and Reduction 85-1992 Image Dynamics Corp.) (Figure 1). PCR of the products of reverse-transcriptase reaction in the absence of RNA was used as negative control. The sequence of primers were: WT1 (outer sense primer for exon 7) 5'-GGCATCTGAGACCAGTGAGAA-3', (outer antisense primer for exon 10) (.5'- GAGA GTCAGACTTGAAAGCAGT-3'.), (internal sense primer for exon7) (5'-GCTGTC CCACTTACAGATGCA-3'), (internal antisense primer 10) (5'-TCAAAGCGCCAGCTG GAGTTT-3'); ß.actin (sense primer) (5'-GTGGGGCGCCCCAG-GCACCA-3'); (antisense primer) (5'-GTCCT- TAATGTCACGCACGATTTC-3')[4]; t(4;11) and t(9; 22) primers used as described previously[4,10,11].

Densitometric analysis of gel electrophoresis images was done by Collage 2.0 program with mean intensity mode.

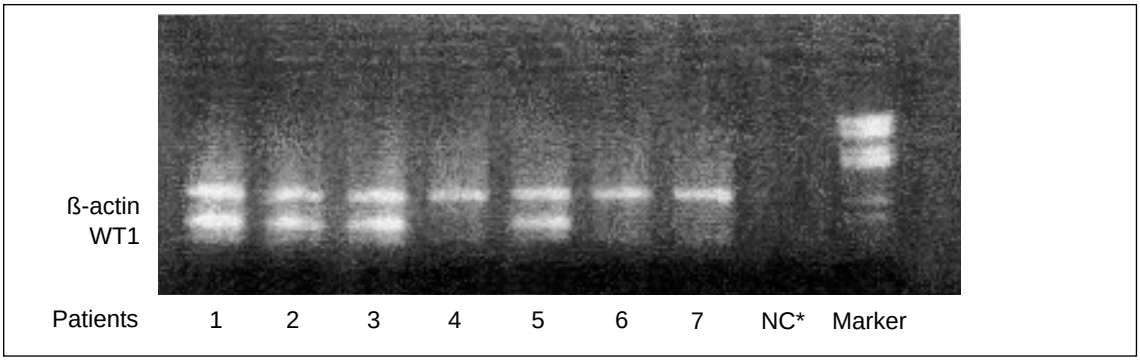

Figure 1. WT1 and ß-actin gene expression in the same gel electrophoresis (WT1 expression is negative in 4th, 6th and 7th patients).

Statistical Analysis: The probability of overall and event free survival was calculated according to the method of Kaplan-Meier. Relationship between WT1 and classic prognostic parameters were calculated with Chi-square and Fisher exact tests.

RESULTS

Frequency and Quantity of WT1 Expression: Twenty-two (78.5%) patients were WT1 positive by second round PCR in bone marrow aspirates at diagnosis and one patient at the end of the induction treatment. Incidence of WT1 gene expression was similar at different ages (< 2 years, 2-9 years and > 9 years). There was also no difference between boys and girls for WT1 expression. Hemoglobin, platelet and leukocyte counts were similar in WT1 positive and negative patients. There was no correlation between WT1 positivity and translocations 4; 11 or 9; 22. There was a statistically significant difference between WT1 positivity and negativity in T cell surface marker positive patients (Table 1). WT1 gene expression levels of patients are shown in Table 2).

WT1 Gene Expression and Response to Treatment: Two patients died during induction therapy due to infection and twenty-six patients (92.9%) achieved CR. The CR rate was almost identical in WT1 positive and negative patients (Table 3). There was no difference in the 40 months OS and EFS between WT1 positive and negative patients (Table 3, Figure 2).

DISCUSSION

Wilms' tumor 1 gene has been shown to be expressed in majority of patients with acute leukemia[2,12-14] and it is downregulate during cellular differentiation[15]. It has also expressed in normal CD 34+ hematopoietic progenitors[16].

Our results were similar to previous studies reporting that WT1 gene is frequently expressed in leukemic blasts[4]. The prevalence of WT1 positivity (78.5%) in our study was comparable with others, which were 72.3% and 73%[6,17]. We were not able to find a correlation between WT1 gene expression and other prognostic factors of childhood ALL such as age, sex, cell counts, translocations 4; 11 and 9; 22, FAB types excluding T cell surface markers positivity. Contrary to our findings, Gaiger et al reported that WT1 gene expression at diagnosis were frequently in patients with high leucocyte number and peripheral blast cell counts, and there was no correlation between WT1 gene expression and immune phenotype[18]. Relationship between WT1 positivity and T cell ALL needs confirmation with further studies, which will consist of large number of T-ALL patients. In the literature, relationship between WT1 gene expression and CR rate is controversial. Inoue et al (4) have reported that CR rate was higher in patients with acute leukemia that have low WT1 gene expression (below 0.6) whereas Schmid et al reported that there was no relationship between WT1 expression and CR rate[6,18]. We studied relationship between different levels of WT1 gene expression and CR rate or EFS, our data suggest that there was no correlation betwe-

Table 1. Characteristics of patients and WT1 gene expression

Characteristics	All patients n= 28	WT1-negative n= 22	WT1-positive n= 6
Age (year)			
Mean ± SD*	5.98 ± 3.52	7.12 ± 3.91	8.14 ± 2.53
Range	0.9-13	0.9-13	3.5-10.1
Sex (%)			
Male	20 (71.5)	16 (72.7)	4 (66.7)
Female	8 (28.5)	6 (27.3)	2 (33.3)
Leukocyte count (/mm^3)			
Mean ± SD	40 396 ± 52 055	36 342 ± 50524	52 558 ± 58 779
Range	1200-171 000	1200-158 000	1900-171 000
Hemoglobin (g/dL)			
Mean ± SD	7.77 ± 2.61	7.53 ± 2.81	8.49 ± 1.87
Range	3.0-12.9	3.0-12.9	5.9-10.1
Platelet count (/mm^3)			
Mean ± SD	95 037 ± 92 024	106 400 ± 101 316	62 571 ± 50 301
Range	11 000-294 000	11 000-294 000	21 000-148 000
FAB type (%)			
L1	20 (71.5)	15 (68.2)	5 (83.3)
L2	6 (21.4)	6 (27.3)	0 (0)
L3	2 (7.1)	1 (4.5)	1 (16.7)
T-ALL (%)	8 (28.6)	7 (31.8)	1 (16.7)
Translocations (%)			
T(4; 11)	3 (10.7)	2 (9.1)	1 (16.7)
T(9; 22)	2 (7.1)	1 (4.5)	1 (16.7)

*= Standard deviation

Table 2. WT1 gene expression levels

WT1/ß-Actin	Patients number
Negative	6
0.5	22
0.6	21
0.8	19
1.0	14
1.2	6
1.4	2

en WT1 gene expression levels and CR rate or EFS which is consistent with the report of Schmid et al. Relationship between WT1 expression and prognosis of leukemia has been investigated by other authors and their conclusions are also controversial. Some studies revealed that high WT1 expression was associated with worse long-term prognosis (4,19) whereas another study has revealed that WT1 expression had no prognostic significance in acute leukemia[6]. We could not confirm the previous observations suggesting that WT1 gene expression has a prognostic value in acute leukemia.

As a conclusion, our results suggest that WT1 expression does not predict for CR and EFS or OS, and the determination of WT1 gene expression at diagnosis has currently no practical significance in the management of patients with childhood ALL.

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Table 3. WT1 gene expression and response to induction treatment

Response to therapy	All patients n= 28	WT1 positive n= 22	WT1 negative n= 6
CR (%)	26 (92.9)	20 (90.9)	6 (100)
No response (%)	0 (0)	0 (0)	0 (0)
Early death (%)	2 (7.1)	2 (9.1)	0 (0)
EFS (%)	75.0	72.8	83.3
OS (%)	78.6	77.3	83.3

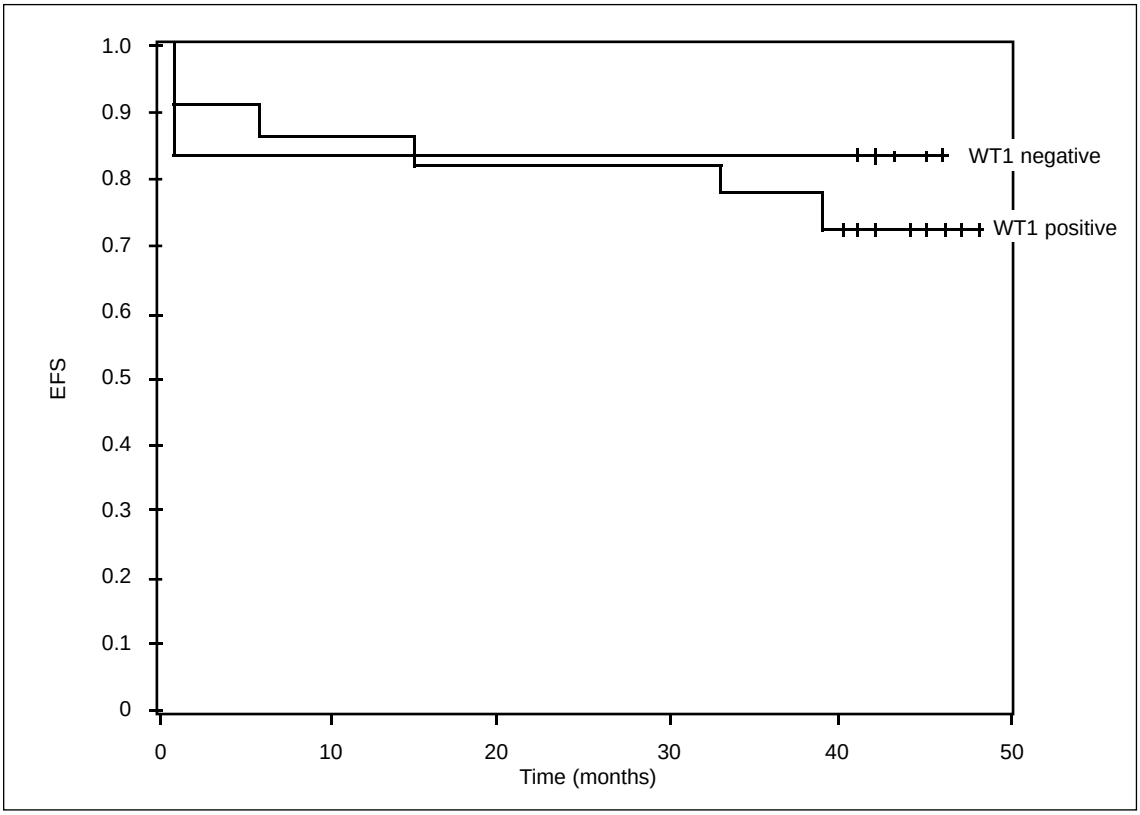

Figure 2. Event free survival according to WT1 gene expression.

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